Scientific Abstract

CML is a malignant disease of the human hematopoietic stemcell characterized by the BCR-ABL fusion gene. Presence of the BCR-ABL encoded $P210^{BCR-ABL}$ tyrosine kinase is required and sufficient to cause malignant transformation. Allotransplant is the only therapy that can cure 40-70% of patients. Because the majority of patients is not eligible for allotransplant, autografts with blood or marrow cells enriched in BCR-ABL$^-$ cells have been performed. Although improved survival may be seen in the chronic phase of the disease when compared with conventional therapy, almost all patients relapse because of persisting disease in the graft and the host. Thus, ongoing post-transplant therapy that eliminates further persisting disease, such as long-term administration of low doses of chemotherapy, will be needed to improve disease free survival after transplant. We have constructed a retroviral vector that contains a double copy antisense (AS) sequence directed at the b3a2-BCR-ABL mRNA breakpoint and a tyr22-dihydrofolate reductase (DHFR) Methotrexate resistant (MTX^R) gene, termed LasBD. LasBD reduces BCR-ABL mRNA and $p210^{BCR-ABL}$ protein in cell lines containing the b3a2-BCR-ABL cDNA and primary b3a2 BCR-ABL CML cells 6-10 fold, but not $p190^{BCR-ABL}$ containing cells. This leads to the restoration of "normal" function of BCR-ABL cDNA$^+$ cells: normal response to cytokines, restoration of apoptotic cell death and restored expression/function of adhesion receptors. Further, LasBD eliminates *in vivo* tumorigenicity of b3-a2-BCR-ABL containing cells by at least three logs. Finally, LasBD renders NL and CML CD34$^+$ progenitors MTX^R. We hypothesize that transplantation of PBPC transduced with LasBD will allow post-transplant administration of MTX to eliminate untransduced Ph$^+$ cells that remain in the host and the graft, while selectively sparing transplanted LasBD transduced Ph$^-$ cells. Because LasBD will also be introduced in some transplanted Ph$^+$ cells, we hypothesize that the AS sequence present in LasBD may suppress $P210^{BCR-ABL}$, rendering Ph$^+$ but BCR-ABL mRNA$^-$ cells functionally normal. The AS sequence would thus prevent selective expansion of transplanted LasBD containing Ph$^+$, MTX^R cells. We propose to conduct a phase I/II clinical study in which PBPC from chronic phase CML patients, with a b3a2 BCR-ABL breakpoint, will be transduced with LasBD and patients treated with MTX after transplant, to determine (1) the toxicity of transducing CD34$^+$ cells with LasBD; (2) the toxicity of administering MTX after autotransplant with LasBD transduced PBPC; (3) the transduction efficiency of Ph$^-$ and Ph$^+$ progenitors with LasBD; (4) the long-term expression of the tyr22-DHFR gene and its effect on the *in vivo* selection of transduced cells; (5) the efficacy of MTX at eliminating untransduced Ph$^+$ cells present in the graft and host; (6) the long term expression of the AS component, its effect on BCR-ABL mRNA, protein and on the behavior of Ph$^+$ cells *in vivo* and *in vitro*.